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Emerging Medicinal Properties of Calotropis gigantea – A Review

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Abstract: *Calotropis gigantea, commonly known as giant milkweed or crown flower, is a ubiquitous weed that thrives along roadsides throughout the Indian subcontinent, encompassing the geographical regions of India, Pakistan, and Bangladesh. This plant has been an integral part of traditional medicine systems for centuries, demonstrating remarkable therapeutic potential through its antimicrobial, antioxidant, and anti-inflammatory properties across various health conditions. In the face of the growing global challenge of antimicrobial resistance (AMR), the demand for novel, naturally derived compounds has increased exponentially. This urgent need has sparked renewed interest in plants like C. gigantea as potential sources of new antimicrobial agents. This comprehensive review aims to collate and analyze the findings from numerous studies conducted on C. gigantea, focusing on four key areas of research: antimicrobial activity, antioxidant properties, toxicity profile, and wound healing capabilities. By examining the plant's efficacy against various pathogens, its capacity to neutralize harmful free radicals, assessing the safety and potential side effects of its derived compounds, and exploring its potential in promoting tissue repair and regeneration, this review seeks to provide a holistic understanding of C. gigantea's pharmacological properties. Through this synthesis of available scientific evidence, the review aims to elucidate C. gigantea's potential applications in modern medicine, particularly in addressing the pressing issue of AMR and developing novel therapeutic strategies.*

Keywords: Antimicrobial Resistance, Antioxidant Activity, *Calotropis Gigantea*, Wound Healing.

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INTRODUCTION

Antimicrobial resistance (AMR) has become a situation of great concern for both healthcare professionals as well as researchers as it poses a direct threat to human's wellbeing and the health of the ecosystem (*Shah P. M.* 2005) therefore, the need to observe novel antimicrobial compounds is the of the utmost priority these days. Plant's various parts having the properties of antimicrobial and anti-inflammatory

compounds promise good results according to a variety of different studies conducted. Since the past, during the dawn of human civilization, plants have been used extensively as traditional medicine. One such medicinal plant is the giant milkweed, crown flower (*C. gigantea)* commonly known as "Aak" in the northern part of the Indian subcontinent, which gets its name from the presence of Calotropin (Figure 1). This weed is frequently found invading field land, on the roadside, alongside buildings, etc. Traditionally used in Indian medicine and it has been the topic of many scientific research studies which showed its potentially usefulness as antimicrobial, pregnancy interceptive, insecticidal, anti-inflammatory, etc. properties (*Rajamohan, S.*, et al 2014). Scientific classification of *C. gigantea* is presented in Table 1.

Figure 1. *C. gigantea [Milkweed] Growing on roadside in India*

Table 1. Systematic classification of C. gigantea

Rank	Scientific name and common name
Kingdom	*Plantae*
Subkingdom	*Tracheobionta – vascular plants*
Super division	*Spermatophyta – seed plants*
Division	*Magnoliophyta – flowering plants*
Class	*Magnoliophyta - Dicotyledons*
Subclass	*Asteridae*
Order	*Gentianales*
Family	*Apocynaceae*
Subfamily	*Asclepiadaceae Borkh. – Milkweed family*
Genus	*Calotropis*
Species	*Calotropis gigantea*

Historical uses of *C. gigantea*

C. gigantea plant is pantropical and has been used since centuries, Indian and along with China, Bangladesh, Malaysia, Pakistan, and other Asian communities employed the plant as a dart poison, for antimicrobial activity, wound healing, livestock disease treatments, for harvesting of fiber from the stem of the plant to religious offering of its flowers to gods, etc. Tribal and historical knowledge has not been properly documented and needed validation via scientific experiments. In the past 2 decades, extensive scientific research has been undertaken, uncovering the useful actions of the *C. gigantea* and its various compounds present in this. This recent research is exploring *C. gigantea* as a potential compound towards therapeutic agents and combat ever emerging trends like anti-microbial resistance. *C. gigantea* is significantly employed in Ayurveda, Siddha and Unani for various purpose (Table 2) (*Pophale, S.*, et al 2023).

Table 2. Application of C. gigantea in different branches of medicine.

Branch of medicine	Parts of the plant used	Ailments/Sickness
Ayurveda	Leaves, flowers, root bark	Paralysis, swellings, and sporadic fevers, asthma, catarrh, anorexia, helminthic infections, inflammations, fever, ascites, intestinal worms, and skin infections

Siddha	Leaves, root, flowers	Deadly snake bites, recurrent fever, intestinal worms, abscess, dental issues, rat bites, swellings, gonococcal arthritis, rheumatic ailments and bronchial asthma
Unani	Root bark	Relief in diarrhoea and dysentery

Effect of *Calotropis gigantea* on various conditions

The medicinal potential of *Calotropis gigantea* has been extensively studied, revealing its significant therapeutic properties across a variety of conditions. Some studies demonstrated that the methanolic extract of *Calotropis gigantea* exhibited broad-spectrum antimicrobial activity against various bacterial and fungal strains. The study attributed this activity to the presence of bioactive compounds such as flavonoids and cardenolides. In-depth analysis of the antimicrobial properties of *Calotropis gigantea* latex, revealing significant efficacy against pathogens like *S. aureus* and *C. Albicans* (*Shah P. M.* 2005 and *Rajamohan, S*., et al 2023). The antimicrobial activity was primarily linked to the latex's ability to disrupt microbial cell walls and inhibit enzyme activity. Some studies evaluated the cytotoxic effects of *Calotropis gigantea* extracts on human cancer cell lines, including breast, colon, and liver cancer cells. The findings indicated that the extracts could induce apoptosis and inhibit cell proliferation, highlighting their potential as anticancer agents (*Hasballah, K*., et al 2018). Key compounds like calotropin and calactin were identified as responsible for the cytotoxic effects (*Hasballah, K*., et al 2018). Few investigators assessed the wound healing properties of Calotropis gigantea leaf extract in rats (*Deshmukh, P. T*., et al 2009). The study found that the extract significantly accelerated wound closure and tissue regeneration, possibly due to enhanced collagen synthesis and its antimicrobial properties (*Pophale, S*., et al 2023 and *Patel, S*., et al 2012) Confirmed the wound healing efficacy of Calotropis gigantea latex in diabetic rats. The study suggested that the latex could improve wound healing by promoting fibroblast proliferation and angiogenesis (*Pophale, S*., et al 2023 and *Deshmukh, P. T*., et al 2009). Here are some key findings from research studies on the plant's pharmacological activities, mechanisms of action, and bioactive compounds (Table 2).

Author, year of publication	**Title**	**Highlights of the Article**
Shah *et al., 2005*	The need for new therapeutic agents: what is in pipeline?	Acknowledgment and highlighting of the increasing phenomenon of anti-microbial resistance and the need to find new therapeutic agents.
***Rajamohan* et al., 2014**	Antioxidant, Antimicrobial activities and GC-MS analysis of Calotropis gigantea white flowers	Evaluation of antioxidant and anti-microbial activities showing promising scope.
***Pophale* et al., 2023**	Review Article on Calotropis Gigantea	Review of phytochemical constituents, anti-inflammatory, wound healing and analgesic activities of Calotropis gigantea.
***Patel* et al., 2012**	Evaluation of antimicrobial activity of C. Gigantea extracts on two main skin infection causing bacteria - *Escherichia Coli* and *Staphylococcus aureus*	Ethanolic extract of *Calotropis gigantea* manifested inhibition property when investigated on both gram-positive *S. aureus* and gram-negative *E. coli.*

***Kumar, G.,*et al 2010**	Antibacterial activity of aqueous extract of *Calotropis gigantea* leaves – An i*n-vitro* study	Evaluation of polar solvent extract in the form of aqueous extract of *Calotropis gigantea* on 6 clinical strains with highest inhibition of *E. coli* and lowest of *K. pnuemoniae.*
***Alam, M. A.*, et al 2008**	Antimicrobial activity of akanda (*Calotropis gigantea L.*) on Some pathogenic bacteria	Anti-microbial evaluation with focus on weak economy countries and comparison of anti-microbial activity of *Calotropis gigantea* with *Wedelia calendulace* showing comparatively better inhibition of the tested organisms.
***Joshi* et al., 2010**	Phytochemistry and evaluation of antioxidant activity of whole plant of *Calotropis gigantea linn.*	Evaluation of antioxidant activity using DPPH Method, Nitric Oxide Method, reducing power method all showed reasonable results.
***Gulchin* et al., 2023**	DPPH Radical Scavenging Assay	DPPH as an evaluation tool in antioxidant profile analysis and its limitations.
***Bauer* et al., 1996**	Antibiotic susceptibility testing by a standardized single disk method	Establishment of a standardized method for testing antibiotic susceptibility.
***Deshmukh* et al., 2009**	Wound healing activity of *Calotropis gigantea* root bark in rats	Determination of toxicity and In-vivo evaluation of *Calotropis gigantea* extract in wound healing with comparison of control (untreated) and Povidone-Iodine showed positive and accelerated wound healing effect.
***Hasballah* et al., 2018**	Acute toxicity and hepatotoxicity evaluation of methanol extract of root bark of *Calotropis gigantea* in rats	Determination of toxicity of methanol extract of root bark of *Calotropis gigantea* acutely and on liver cells upto 5000mg/kg of body weight in Wistar rats concluded no toxic effect but going above this dose can result in hepato-toxic damage.

Preparation and Methods for Extraction

Researchers have used several different methods for the preparation of extracts mainly involving inorganic solvents Ethyl Alcohol, Methyl Alcohol, Chloroform, Hexane etc. (*Patel, S.*, et al 2012; *Kumar, G.,*et al 2010; *Alam, M. A*., et al 2008). The general workflow used for the extraction is presented in figure 2. Additionally, Methods used to test effect of plant using different methods are shown in figure 3.

The commonly used protocol in most of the studies is described as follows:

a) The plant parts be it leaves, flowers, root or bark were obtained and authenticated from botanists
b) After authentication, the samples are washed with distilled water

c) After washing, the samples are sun dried or oven dried or even air dried if sensitive to high temperatures
d) The dried mass is then grinded into a powder and that powder is taken by weight in a solvent of choice by the researcher according to weight by volume ratio, for example 5gm dried powder in 45ml solvent
e) Mixed through a shaker and filtered
f) The filtrate is then concentrated using Soxhlet or rotating evaporator till just a bare minimum amount of moisture remains
g) Stored at 4 degree Celsius till further use
h) Working solution is prepared according to need and design of the study

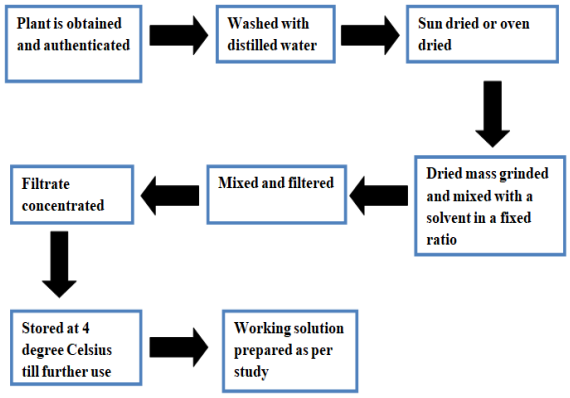

Figure 2. Workflow of extract preparation

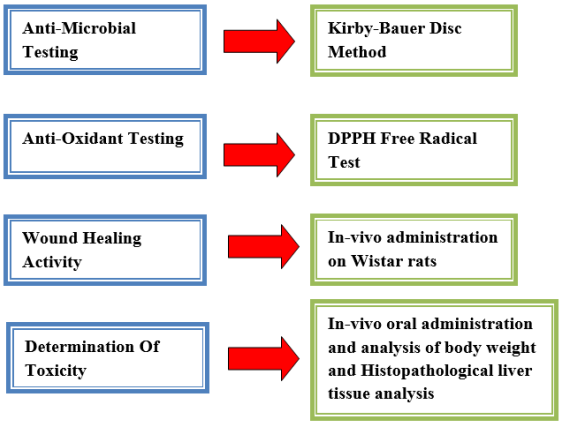

Figure 3. Methods used to test different activities

Testing of Antioxidant Properties

The evaluation of antioxidant properties of pharmaceutically significant compounds is done using the DPPH Free Radical Scavenging Method. Using this assay the IC50 value, which is the minimum inhibitory concentration required to scavenge 50% of DPPH free radicals in a solution by the sample that is being tested. This was found out to be 54.29µg/ml of whole plant ethanolic extract of *Calotropis gigantea* and control of ascorbic acid at 18.21µg/ml (*Amit Joshi, A. J.,* et al 2010). The principle behind the assay is the bleaching of DPPH (1, 1- diphenyl-2-picrylhydrazy) prepared in the methanol base which is purple in color which is used as a measure to assess the hydrogen or electron donating capacities of compounds (Gulcin, İ., & Alwasel, S. H. 2023). The solution prepared at different concentrations mixed with DPPH, and enabled to stand at room temperature for 30 minutes then analyzed at 515nm in spectrophotometer further the percent of inhibition of DPPH reduction (decolorization) is measured by:

$$\% \text{ of inhibition} = [A_0 - A_{sample}]/ A_0 \times 100$$

Where (A_0) is absorbance of control (blank) and (A_{sample}) is absorbance of test compound. The IC50 is calculated from the plot of inhibition percentage against sample concentration. It was observed that the chloroform extract of C. gigantea has the highest scavenging activity (*Rajamohan, S.*, et al 2014).

Anti-Microbial Evaluation

The zone of inhibition Kirby Bauer disc method is the most widely used procedure to test the antimicrobial properties of samples of interest. Mueller Hinton Agar is autoclaved at 15lbs of pressure at 121 degree Celsius for 20 minutes then poured on sterile petri dishes and allowed to cool down. A bacterial culture of 0.5McFarland Standard turbidity is swabbed on the media using sterile cotton swabs. Sterile discs containing the sample of interest and Antibiotics that are used as positive control are placed on the agar surface. The samples are incubated for prescribed durations according to the cultures taken. The zone of inhibition, marked by a clear zone around the placement of the antibiotic disk

and the sample disc (if present) is measured by a clear ruler in mm (*Bauer, A. W.,* et al 1966)

In studies conducted, both aqueous and non-aqueous extracts of *Calotropis gigantea* have displayed positive antimicrobial results. The aqueous extract was prepared by washing, drying and blending powdered leaves and adding the 10gm leaf powder in 100 ml sterile distilled water, which was concentrated using orbit shaker at 120 rpm for 24 hours then obtained the filtrate through Whatman filter paper, the filtrate was further concentrated by using rotary and concentrated using a lyophilzer. The dried *Calotropis gigantea* plant extract was dissolved in sterilized distilled water to make 1000µg/ml solution. Pure cultures of selected bacteria including *S. aureus, K. pneumoniae, B. cereus, P. aeruginosa, M. luteus* and *E. coli* were tested. Positive and negative control was done by taken antibiotics: Amoxycillin (10µg/disc) for *B. cereus* and *K. pneumoniae*, Penicillin G disc (10 µg/disc) for *S. aureus* and *M. luteus* and Polymyxin-B (10 µg/disc) for *E. coli and P. aeruginosa*, in the meantime autoclaved distilled water was employed as negative control. The results were calculated in terms of Relative Inhibitory Percentage with respect to positive control were as follows: The aqueous extract showed highest relative percentage inhibition against *B. cereus* 188.52 % followed by *E. coli* 167.47%, *P. aeruginosa* 105.19%, *K. pneumoniae* 77.63%, *S. aureus* 46.04% and *M. luteus* 24.92% (*Kumar, G*., et al 2010).

Studies on non-aqueous extract involving chloroform, ethyl acetate, ethanol and methanol have also shown positive results. In a 2014 study, concentration of 50µl of 10 µg / ml of sample and 10 µg / ml of positive control drugs Ciprofloxacin and Amphotericin B taken as common for test organisms *E. coli, S. aureus* and *K. pneumonia* and *A. niger, A. flavus* and *C. albicans* gave the following result: Chloroform and ethyl acetate extracts showed largest zone of inhibition against *E. coli* while the methanol extract showed significant inhibitory activity against *E. coli.* Chloroform extract showed higher activity of inhibition zones against *S. aureus* followed by ethyl acetate extracts inhibition zones against *S. aureus*. However, methanol extract showed maximum inhibition zone against *S. aureus*. Both chloroform and ethyl acetate extract manifested greater activity than methanol extract. Out of the investigated organisms, *S. aureus* is highly vulnerable followed by *E. coli*. The antimicrobial activity of plant extracts is more effective on Gram-positive organisms as compared to Gram-negative bacterial. The anti-fungal test observed that the chloroform and ethyl acetate extracts were more sensitive to *C. albicans* as compared with *A. niger* and *A. flavus*. The results found that the chloroform, ethyl acetate and methanol extract of *C. gigantea* were found to be active against all the microbes investigated (*Rajamohan, S*., et al 2014)

In a 2022 study, the *C. gigantea* plant extract was dissolved in ethanol, which was sterilized using a Millipore filter and then loaded onto a sterile filter paper disc. Ten ml of agar medium was poured into a sterile Petri plate, then 15 ml of seeded medium earlier inoculated with bacterial suspension to achieve 10^5 CFU per ml of medium. Gentamycin was employed as a positive control. Further the sterile filter paper discs loaded with plant extract concentrations of the different selected concentrations were placed on top of Mueller-Hilton agar plates. Filter paper discs loaded with 5 mg of Gentamycin used as a positive control. The plates were placed in the refrigerator at 5° Celsius for two hours to diffuse the extract, then incubated at 35° Celsius for 24 hours. Inhibition was measured. Ethanol extract showed larger zone of inhibition for *S.aureus* as compared to *E.coli* at 50µg concentration.

In-vivo wound healing activity

The wound healing effects of ethanolic extract of *Calotropis gigantea* was evaluated in an excision wound model by taking a 5% weight by weight ointment in a simple base. Animals were placed into 3 groups. Group 1 considered as not treated with anything and kept as control, group 2 considered as the test standard with 5% percent povidone-iodine treatment and group 3 considered as test, treated with *C. gigantea* extract. For testing of incision wound model, dosages were set at group 2 on 100mg/kg, group 3 on 200mg/kg and group 3 on 400mg/kg applied daily. Group 1 was kept as control and group 5 was standard for treatment with 50mg/kg povidone-iodine. Results showed significant increase in wound healing activity as compared with control in all the studied models by analysis of markers studied (*Deshmukh, P. T.,* et al 2009).

Determination of Toxicity

In an experimental study 2018, methanol extract of *Calotropis gigantea* was tested on Wistar rats to identify its severe toxicity and hepato-toxicity. The tested animals were acclimatized for 1 week in the experimental conditions and allowed to settle in. Single dose at concentrations of 1250mg/kg, 2500mg/kg and 5000mg/kg was administered orally by gavage needle and a control group of rats, without any dosing were kept for comparison of results. Acute toxicity can be simply determined by reduction in body weight and reduction in weight of body organs. No significant difference in body weight was found between the test animals given the plant extract and the control rats which were not administered any dosage, thus showing no inhibition of growth. For the testing of hepato-toxic effects, microscopic analysis of the liver after sacrifice was done at 400 x magnification, cell destruction or damage was classified into parenchymatous degeneration, hydropic degeneration and necrosis. Even though the extract was determined to be nontoxic due to no mortality but histopathological, effects on parenchymatous deterioration on dosage of 1250 mg/kg, hydropic degeneration on dosage 2500 mg/kg, and hepatic necrosis on 5000 mg/kg were observed indicating that the extract can be damaging in doses above 5000mg/kg (*Hasballah, K*., et al 2018)

Conclusion

The inclusion of *Calotropis gigantea* in folk medicine and tradition medicine for curing of a variety of ailments is now backed with scientific proof. The antioxidant properties tested with DPPH scavenging assay and antimicrobial assay using Kirby Bauer Disc method on MHA agar using numerous studies on both aqueous and non-aqueous extracts have shown positive results and shed light towards a new direction of therapeutic agents, which is the need of the hour in the emerging trend of antimicrobial resistance spreading and costing lives at an alarming rate. *Calotropis gigantea* also displays promising scope of results in wound healing activity and its anti-microbial effects can be clubbed to prevent infection of wounds along with acceleration of rate of healing. Toxicity studies show that alcohol extracts of Calotropis gigantea are safe and nontoxic till certain specified limits as displayed by in-vivo studies of Wistar rats, however organ toxicity and exposure dose, frequency and duration are critical factors and must be extensively studied and monitored to ensure safety for human trials and use. Calotropis gigantea looks a very promising plant with amalgamation of useful compounds and properties that has the potential to be become a large-scale solution for ailments.

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